Consensus on the standardization of terminology in thrombotic thrombocytopenic purpura and related thrombotic microangiopathies.

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Essentials:

- **An international collaboration provide a consensus for clinical definitions**
- **We standardize terminology in TMAs and thrombotic thrombocytopenic purpura.**
- **We define TTP, differential diagnosis, monitoring disease and response to treatment.**
- **We describe requirements for ADAMTS13 assays for diagnosis and follow up of TTP patients.**

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Abstract:

Background Thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS) are two important acute conditions to diagnose. Thrombotic Microangiopathy is a broad pathophysiological process that leads to microangiopathic hemolytic anemia , thrombocytopenia and involves capillary and small vessel platelet aggregates. The most common cause being disseminated intravascular coagulation (DIC), which may be differentiated by abnormal coagulation. Clinically, a number of conditions present with microangiopathic hemolytic anemia and thrombocytopenia (MAHAT), including cancer, infection, transplantation, drugs, autoimmune disease and pre eclampsia (PE) and HELLP (Hemolysis, Elevated Liver enzymes, Low Platelet count) syndrome in pregnancy. Despite overlapping clinical presentations, TTP and HUS have distinct pathophysiology and

treatment pathways. **Objectives:** Presented is a consensus document from an international working group on TTP and associated TMAs (thrombotic microangiopathies). **Methods:** The international working group has proposed definitions and terminology based on published information and consensus based recommendations. **Conclusion:** The consensus aims to aid clinical decisions but also future studies and trials, utilizing standardized definitions. It presents classification of the causes of TMA, and criteria for clinical response, remission and relapse of congenital and immune mediated TTP.

Introduction

The elucidation of the pathophysiology of TTP and HUS, in the past 20 years, has transformed our understanding of the phenotypes, genotypes and therapies for these life-threatening conditions. Work on standardization has been addressed [1], but this document aims to develop robust criteria for future clinical use, studies and trials. Clinical and pathophysiologic features of TTP, atypical Hemolytic Uremic Syndrome (aHUS) and disorders with similar presentations, their investigation and subsequent management vary. This consensus document aims to rationalize and standardize definitions. Conditions often included in the initial differential diagnosis of TTP are discussed. Definitions for remission, refractory and relapsing disease are defined. ADAMTS 13 (***a d****isintegrin* ***a****nd* ***m****etalloproteinase with a* ***t****hrombo****s****pondin type 1 motif, member* ***13***) assays are central to diagnosis and are discussed. We therefore also describe the minimum requirements for validation of current and future assays.

Methods:

The development of this document involved key international, primarily clinical, experts in TTP and related TMAs. The concept was supported by the European Hematology Association, scientific working group (EHA-SWG), platelet group, who have endorsed this collaboration. Members of the group have met at key international meetings, including EHA, International Society of Thrombosis and Hemostasis (ISTH) and American Society of Hematology

(ASH) and all versions of the document have been reviewed and edited by the authors. Articles were identified by a computer-assisted search of the literature published in English using the National Library of Medicine PubMed database. The authors also undertook a focused review of the available literature. Where there was a lack of robust evidence, the international working group concluded a consensus-based approach was preferable. The conclusions are relevant to both children and adults.

Thrombotic Microangiopathy

The term TMA is a pathological term used to describe occlusive microvascular or macrovascular disease, often with intraluminal thrombus formation, but is also defined clinically by microangiopathic hemolytic anemia and thrombocytopenia (MAHAT). This does not specifically define a condition; therefore further investigations are required to identify the underlying cause for the presentation of a TMA, including TTP (Figure 1).

Pathological Features of TMAs

A number of different pathological entities have been identified, including:
(i) von Willebrand factor (VWF)-platelet thrombosis; seen with severe ADAMTS13 deficiency related to anti-ADAMTS13 antibodies or a congenital absence of ADAMTS13, the hallmark of TTP. The characteristic pathology of TTP is the presence of ultra large VWF multimers and VWF and platelet rich thrombi in arterioles and capillaries[2, 3]. Presentation with large vessel thrombosis (stroke or myocardial infarction) is believed to result from vascular injury caused by thrombosis of the vasa vasorum

(ii) Fibrin-platelet thrombosis; commonly seen in disseminated intravascular coagulation (DIC) [4] but occasionally seen in catastrophic antiphospholipid syndrome (CAPS), heparin induced thrombocytopenia (HIT), and HELLP syndrome.

(iii) Thrombotic Microangiopathy; characterized by endothelial swelling or disruption associated with hyalinosis or fibrinoid necrosis, usually without an inflammatory cell infiltrate. Chronic TMA is associated with myointimal proliferation resulting in concentric rings of cells and matrix surrounding small vessels (sometimes termed onion skinning), which can be indistinguishable from the effects of chronic hypertension. Intraluminal thrombosis is frequently present, but is not required for the diagnosis [5]. Examples include MAHAT caused by shiga toxin, complement dysregulation and drugs (eg gemcitabine).

(iv) Vasculopathy/vasculitis; changes involve not only endothelial cells and intima, may be autoimmune (e.g. lupus vasculitis [6], scleroderma renal crisis [7]) or infectious (e.g. Rocky Mountain spotted fever [8] , viraemia, fungemia). An inflammatory component may be evident.

(v) Intravascular clusters of cancer cells (tumor cell embolism) may occur in patients with advanced cancer [9].

Clinical features of TMAs

Peripheral blood film features consistent with MAHA include fragmented red blood cells (schistocytes), polychromasia and anemia. Quantification of fragmentation/schistocytes is not reliably available and remains subjective. Laboratory evidence of hemolysis includes elevated lactate dehydrogenase (LDH), reticulocytosis, low/absent haptoglobin, and a negative direct antiglobulin test. Thrombocytopenia will be evident in the complete blood count (CBC) and on the blood film [10].

Thrombotic thrombocytopenic purpura (TTP)

Definition of TTP: **MAHA with moderate or severe thrombocytopenia, with associated organ dysfunction, including neurologic, cardiac, gastrointestinal and renal involvement, although oliguria or anuric renal failure requiring renal replacement therapy is not**

typically a feature [10, 11]. TTP is confirmed by a severe deficiency (<10%) of ADAMTS13 activity [2, 3]

TTP is recognized as a multi-organ process with variable clinical features. Anemia may not be immediately obvious. Thrombocytopenia is generally severe (platelet count <30 x10^9/L), but higher platelet numbers do not exclude the diagnosis.

Presentation with renal failure requiring renal replacement is not a common feature of TTP. However, occasionally patients with multi organ failure may develop an acute kidney injury requiring renal support. Congenital TTP (cTTP) patients may present with acute renal failure [12]. cTTP cases may previously have been misclassified as HUS [13]

Acute TTP presentations may include bleeding symptoms such as bruising, hematuria or thrombotic symptoms associated with neurological or cardiac involvement. In practice, TTP is suspected in:

- Isolated MAHAT
- New focal neurological symptoms, seizures or myocardial infarction (MI), with unexplained MAHAT
- Prior history of TTP

<u>Confirming a diagnosis of TTP:</u>

Current ADAMTS 13 activity assays provide levels of sensitivity <5% or 1%. However, for the diagnosis of TTP, ADAMTS 13 activity levels < 10% are diagnostic. This is to include antibody mediated cases and cTTP, including late onset cTTP.

The diagnosis of TTP should be confirmed with ADAMTS 13 activity performed on a sample taken prior to initiation of therapy, specifically plasma-

based. Samples taken immediately following plasma therapy may give a falsely raised ADAMTS 13 activity. The presence of anti-ADAMTS 13 IgG antibodies may help confirm TTP in these situations. However, in autoimmune TTP, >75% of patients exhibit <10% ADAMTS 13 activity immediately before the next therapeutic plasma exchange session for several days after starting daily exchange therapy [14].

Assays at diagnosis should include: ADAMTS 13 activity, functional inhibitor based on mixing studies, and/or an anti-ADAMTS 13 IgG. This panel should correctly identify TTP patients in the majority of cases.

Measurement of ADAMTS 13 at regular intervals during treatment and in remission (e.g. weekly during treatment, monthly, then 3 monthly during follow-up period, extending to 6-12 monthly) may provide data in regard to the risk of relapse and persistence of subclinical disease activity [15]. ADAMTS 13 activity <10% with or without a detectable inhibitor is associated with a significant risk of relapse and can guide elective, prophylactic therapy preventing further relapse [16-18] in iTTP.

Congenital TTP **(Upshaw-Schulman syndrome) (cTTP): persistent severe deficiency (<10%) of ADAMTS 13 activity, with no evidence of anti-ADAMTS 13 autoantibodies, confirmed by molecular analysis of the *ADAMTS 13* gene confirming a pathogenic homozygous or compound heterozygous mutational defect.**

Persistent ADAMTS 13 activity <10% with no detectable anti-ADAMTS 13 IgG antibodies (confirmed in the acute phase and in remission after treatment) suggests a diagnosis of cTTP, especially in the following circumstances:

- Neonates presenting with thrombocytopenia, red cell fragmentation on blood film, often with hyperbilirubinemia.
- Children/ adults if there is a familial antecedent of TTP, especially in siblings from consanguineous parents.
- First TTP episode in adulthood, in particular, during pregnancy.

The diagnosis of cTTP is confirmed following mutational analysis [19-21]. Patients can present in the neonatal period, childhood or adulthood, with presentations typically associated with a trigger, such as infection, vaccination, or dehydration [22]. Pregnancy may precipitate acute manifestations in women with cTTP. Presentation of TTP in pregnancy, often associated with fetal loss, particularly in the 2nd trimester, requires exclusion of late onset cTTP [23-25].

The diagnosis is confirmed if pathogenic *ADAMTS 13* mutations are identified. However, many genetic variants are of unknown significance and require expression studies. Infusion of plasma resulting in recovery of ADAMTS 13 activity and the disappearance of ADAMTS 13 activity with a half life of 2-3 days, supports the diagnosis of cTTP [26].

Immune mediated TTP (iTTP):

This defines acquired TTP and can be divided into those cases in which a precipitating cause can or cannot be confirmed. All cases require immediate therapy (TPE and steroids), but differentiating subgroups can help to tailor therapy.

Primary iTTP: This describes acquired autoimmune TTP for which there is no obvious underlying precipitating cause/disease, ADAMTS 13 activity <10% with the presence of ADAMTS 13 autoantibodies.

Primary iTTP accounts for the majority of cases of TTP. The precipitating factor or trigger for primary immune TTP has not been identified, although an underlying genetic risk in Caucasian patients has reported the presence of HLA DQ-7, HLA DRB1*11 & HLA DRB3* [27]

Secondary iTTP: This describes acquired autoimmune TTP for which a defined underlying disorder or trigger can be identified including connective tissue disease (such as systemic lupus erythematosis (SLE)), human immunodeficiency virus infection, cytomegalovirus (CMV) infection and/or a specific precipitating factor

(e.g. pregnancy, drugs). Treatment of the underlying disorder and/or removing the underlying precipitant may be required as well as standard TTP therapy. The presence of severe deficiency (<10%) of ADAMTS 13 activity and ADAMTS 13 autoantibodies confirms a diagnosis of TTP.

Secondary causes include infectious agents such as HIV [28] , drugs, such as ticlodipine, quinine, simvastatin [29, 30], trimethoprim [31] , pegylated interferon [32], as examples. The association is typically rare and idiosyncratic. Pregnancy or certain autoimmune diseases, such as SLE [33], Sjogren's syndrome [34] and rheumatoid arthritis (RA) maybe associated with iTTP. Treatment of the underlying precipitant or stopping implicated drugs in conjunction with acute standard therapy (plasma exchange and immunosuppression eg steroids) may be necessary. In HIV associated TTP, plasma exchange is of value, in conjunction with HAART (highly active antiretroviral therapy) [28, 35]. Secondary iTTP constitutes only a small proportion of the overall cases of TTP.

There remain, however, a number of cases that meet the clinical criteria for TTP, but with ADAMTS 13 activity levels that are not severely deficient and in whom an alternative diagnosis cannot be determined. In these patients treatment with plasma exchange, needs to be individualized and continued only if a clear clinical benefit is obvious. Such cases should be discussed with expert centres in TTP/HUS.

Differential diagnosis of TMA

There are a number of conditions that have presenting features (both clinical and laboratory) similar to TTP. ADAMTS 13 assays are essential in their differentiation **(Table 1).**

Hemolytic Uremic Syndrome (HUS): HUS is defined by MAHAT and renal injury, which is the predominant feature. Thrombocytopenia may not be as severe as in TTP, and anemia at presentation can be variable (Figure 1).

Infection Associated HUS (IA- HUS), often referred to as STEC-HUS describes an infectious etiology associated typically with E. coli 0157:H7, that express Shiga toxin. The TMA presents 5-7 days after the infection with hemorrhagic colitis. E coli 0157: H7 is not the only subtype associated with infection associated HUS. Indeed other subtypes of E coli [36], Salmonella, Shigella, and Campylobacter associated HUS can present similarly.

Complement Mediated HUS (CM-HUS) usually results from defective regulation of the alternative complement pathway [37]. CM-HUS can be triggered by infection (and diarrhea may be evident at presentation), vaccinations, or pregnancy. Heterozygous mutations may be identified, affecting complement regulators including complement factors H (CFH) and I (CFI), complement factor H related protein (CFHR1, 3 and 5), CD46 (MCP), or gain of function mutations in C3 and complement factor B (CFB). CM-HUS associated with anti-complement factor H antibodies (associated with a polymorphic deletion of CFHR1) can also occur, particularly in children, and is responsive to immunosuppressive therapy [38]. CM-HUS is also described associated with defects in other genes, including *THBD*, which encodes thrombomodulin and with bi-allelic mutation of the *DGKE* gene that encodes diacycl glycerol kinase ε, which regulates diacyl glycerol activated Protein Kinase C signaling in endothelial cells. While the pathophysiology of *DGKE*-associated HUS is not completely understood it is not thought to directly involve disruption of complement alternative pathway regulation [39].

Mutational analysis may take weeks to be completed and in 40-50% of cases no mutations are identified [40]. Therefore, treatment decisions must be made on the basis of the clinical presentation and absence of severe ADAMTS 13 deficiency. CM-HUS may respond to plasma exchange but eculizumab, a humanized monoclonal antibody, which binds to and inhibit C5, is more efficacious, except in DGKE-associated disease [41]. In addition, new complement inhibitors are being developed. A C5 polymorphism, described in the Japanese population, is associated with a reduced response to eculizumab [42].

The clinical presentation of CM-HUS can be variable and includes:

- renal failure, MAHAT;
- renal failure with MAHA but a normal platelet count;
- chronic or progressive renal failure, without a history of MAHA;
- severe hypertension, MAHA or thrombocytopenia, with or without renal impairment, often diagnosed by renal biopsy.

In addition to molecular testing, a response to anti-complement therapy may provide confirmation of the diagnosis.

Rare secondary causes have been identified causing HUS [43](Table 1).

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Definition of hematological response to treatment

Clinical Response: Sustained normalization of platelet counts above the lower limit of the established reference range (eg >150x10^9/L) and LDH (<1.5 upper limit of normal (ULN)) after cessation of plasma exchange.

Clinical Remission: Clinical response after cessation of plasma exchange and maintained for greater than 30 days.

Clinical response and clinical remission would be associated with stabilization of parameters if end organ damage is severe or improvement in function with normalization of laboratory parameters.

Exacerbation: reduction in platelet count to below the lower limit of the established reference range (eg<150 x 10^9/L), increased LDH, and the need to restart plasma exchange within 30 days of the last plasma exchange after a clinical response to plasma exchange.

Relapse: Fall in platelet count to below the lower limit of the established reference range (eg <150 x 10^9/L), +/- clinical symptoms >30 days after stopping plasma exchange for an acute TTP episode, requiring re-

initiation of therapy. This is usually associated with a new increase in LDH.

In patients achieving a normal platelet count, a >10% reduction within 24 hours may represent impending exacerbation (during a period of daily plasma exchange therapy or within 30 days of stopping plasma exchange) or relapse and requires close monitoring. Exacerbation or relapse may not demonstrate the full spectrum of clinical features seen in the initial acute TTP presentation. ADAMTS 13 activity will be <10% at exacerbation/relapse, either as a persisting deficiency or a de novo deficiency after transient normalisation.

<u>Refractory TTP:</u> Persistent thrombocytopenia, lack of sustained platelet count increment or platelet counts <50 x109/L and persistently raised LDH (>1.5 X ULN) despite 5 plasma exchange [44] and steroid treatment. This would be defined as SEVERE if the platelet count remained < 30 x 10^9/L.

Normalization of a platelet count is typically above the lower limit of the established reference range (eg >150x10^9/L), however, country/regional variability relating to the 'normal range' should be noted. LDH may not be in the normal laboratory range with clinical response or remission, but decreasing levels with increasing platelet counts is in keeping with clinical response.

The definition of refractory TTP can be challenging. Cases with an initial response, but then a reduced platelet count that is difficult to increase, may be considered refractory and this may occur after 5 plasma exchange procedures. However, the clinical condition is likely to be more stable at this point.

Intensive plasma exchange refers to increased volumes per plasma exchange eg a single volume to 1.5 plasma volume or increased frequency of plasma exchange eg twice daily. Protocols will be institution specific.

During an acute presentation, the definition of refractory disease may require an escalation in therapy. The median time to achieve a clinical response, with daily plasma exchange, is approximately 10-15 days [45]. Patients admitted to an ICU setting with neurological and /or cardiac features may require longer plasma exchange therapy [46]. Patients failing to achieve remission or whose platelet count and LDH initially improve but worsen despite on-going treatment would also be considered to have refractory disease.

Relapsing TTP:

There are a number of identified risk factors that may be associated with or precipitate a relapse of iTTP,

- **ADAMTS 13 deficiency (particularly <10% activity)**
- **Persistent severe ADAMTS 13 deficiency in remission**
- **Persistent anti-ADAMTS 13 antibodies in remission**

or congenital disease

- **Pregnancy**
- **Infection/live vaccinations**
- **Drugs including drugs of abuse eg. cocaine, alcohol**

In iTTP, a severely reduced ADAMTS 13 activity persisting during remission is associated with relapse, particularly when associated with inhibitory autoantibodies [47]. Therapy should be considered to clear antibodies and normalize ADAMTS 13 activity, in patients felt to be at high risk for relapse [16, 18]. Pregnancy [48] , infections and live vaccinations in particular may precipitate an acute episode of cTTP. Often, an initial acute TTP episode, even though occurring only in adulthood, is followed by recurrent episodes [49].

Factors relating to severity/prognosis of TTP

- **Presence of Anti-ADAMTS 13 IgG antibody associated with severe ADAMTS 13 deficiency**
- **Raised troponin at presentation**
- **Presence of neurological features**
- **Older age**
- **Low ADAMTS 13 antigen at presentation**

There are few predictors of disease severity, but necessity of intubation and ventilation, older age, neurological features [50]and cardiac symptoms and/or raised troponin,[51] with high inhibitor levels/IgG autoantibodies to ADAMTS 13 are key predictors. Ethnicity and ADAMTS 13 activity <10% are associated with exacerbation and relapse respectively. Non Caucasians have increased exacerbation and relapse, but reduced mortality. Inhibitor or anti-ADAMTS 13 IgG levels predicting the severity of disease have not been defined [52], but levels (anti-ADAMTS 13 IgG >50% or > 2 BU/ml) are associated with a poorer outcome [51]. Low ADAMTS 13 antigen level at presentation may be associated with worse clinical outcome in iTTP [53]. Routine laboratory parameters such as platelet count, LDH, and hemoglobin are not predictive of severity, although failure to improve with therapy and hence refractory disease is associated with a worse outcome.

ADAMTS 13 assays:

ADAMTS 13 activity: determines the amount of functional ADAMTS 13 via direct or indirect measurement of VWF cleavage. Current methods include FRETS-VWF73, chromogenic VWF-73 [54], chromogenic VWF-73 [55], FRETS-rVWF71 [56] FRET-VWF86 and SELDI-TOF mass spectrometry [57]. A standard curve using pooled normal plasma (PNP) for each assay plus control samples (e.g. plasma of a cTTP patient for an low range sample; the WHO 1st International Standard ADAMTS 13 [58]), which have undergone in-house calibration.

ADAMTS 13 antigen: determines the amount of ADAMTS 13 protein in plasma. Low levels appear to be associated with poor prognosis [53].

Anti-ADAMTS 13 antibodies: usually IgG but IgM and IgA may be also relevant. Anti-ADAMTS 13 antibodies are measured by ELISA, Western blot or immunoprecipitation. ADAMTS 13 antibodies by ELISA may be seen in 5%-10% of healthy individuals however, this may be manufacturer/assay dependent [59]. ELISA or Western blotting using recombinant ADAMTS 13 to detect anti-ADAMTS 13 in patient's plasma can assess the presence of anti-ADAMTS 13 antibodies. In-house or commercially available assays should have appropriate validation and controls to include low, medium and high antibody titres.

ADAMTS 13 inhibitor assays: identifies and quantitates anti-ADAMTS 13 antibodies that functionally inhibit ADAMTS 13 in vitro. Functional ADAMTS 13 inhibitors are assessed in classical Bethesda-like mixing studies. Samples are heat-inactivated for 30 minutes at 56°C to abolish endogenous ADAMTS 13, before mixing, at various dilutions, with PNP. After incubation at 37°C for 2 hours, residual ADAMTS 13 activity is measured and the residual activity transformed in Bethesda units,(1BU/ml of inhibitor inhibits 50% of normal plasma ADAMTS 13). For routine purposes it is usually sufficient to titrate up to 2 Bethesda units/ml.

Factors affecting ADAMTS 13 levels

Severe hemolysis with marked hyperbilirubinemia [60]or severe lipemia may give falsely low ADAMTS 13 activity results, particularly when using fluorogenic detection e.g. FRETS [54]; Free hemoglobin in plasma from intravascular hemolysis can also inhibit ADAMTS 13 activity [61].

There is good concordance between assays in the range of severe deficiency of ADAMTS 13 (defined as either <5% or <10%). However assay conditions can affect results. There are numerous variables in quantification of ADAMTS 13 activity levels among different platforms or when performed in different laboratories. Further studies involving large series of plasma samples are needed to evaluate the newly developed commercial kits.

Pre-analytic aspects: specimen type and acceptability criteria

ADAMTS 13 activity can be assessed in citrated plasma, serum or lithium-heparin plasma. While extensive comparisons between these are missing, small series demonstrate equality [62]. EDTA plasma is not suitable for ADAMTS 13 activity testing, as it is an irreversible inhibitor of ADAMTS 13 activity.

Evaluation of assays

- Detection (sensitivity) limit of the method:

. For ADAMTS 13 activity measurement (defined as the lowest activity that is distinguishable from buffer or heat inactivated plasma respectively): this should be ≤ 5%, but preferably < 1%.

. For the detection of inhibitors: difficult to standardize, but a negative control and at least 2 positive antibody controls, which have demonstrated reproducibility with the technique, should be considered. The detection limit is approximately 0.4-0.6 BU/ml.

- Precision of the method: intra-assay reproducibility (within series), inter-assay reproducibility (between run in one given laboratory and also inter-laboratory precision): should have CVs of <10%.

In conclusion, we have developed definitions for TTP and associated TMAs that we hope will promote uniformity in publications, associated studies and clinical trials. The field is ever expanding and the group will be required to undertake a review again in future years.

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References

1 Sarode R, Bandarenko N, Brecher ME, Kiss JE, Marques MB, Szczepiorkowski ZM, Winters JL. Thrombotic thrombocytopenic purpura: 2012 American Society for Apheresis (ASFA) consensus conference on classification, diagnosis, management, and future research. *J Clin Apher*. 2014; **29**: 148-67. 10.1002/jca.21302.

2 Furlan M, Robles R, Solenthaler M, Wassmer M, Sandoz P, Lammle B. Deficient activity of von Willebrand factor-cleaving protease in chronic relapsing thrombotic thrombocytopenic purpura. *Blood*. 1997; **89**: 3097-103.

3 Tsai HM, Lian EC. Antibodies to von Willebrand factor-cleaving protease in acute thrombotic thrombocytopenic purpura. *N Engl J Med*. 1998; **339**: 1585-94. 10.1056/nejm199811263392203.

4 Levi M, Schultz M, van der Poll T. Sepsis and thrombosis. *Semin Thromb Hemost*. 2013; **39**: 559-66. 10.1055/s-0033-1343894.

5 Skerka C, Licht C, Mengel M, Uzonyi B, Strobel S, Zipfel PF, Jozsi M. Autoimmune forms of thrombotic microangiopathy and membranoproliferative glomerulonephritis: Indications for a disease spectrum and common pathogenic principles. *Mol Immunol*. 2009; **46**: 2801-7. 10.1016/j.molimm.2009.05.018.

6 Song D, Wu LH, Wang FM, Yang XW, Zhu D, Chen M, Yu F, Liu G, Zhao MH. The spectrum of renal thrombotic microangiopathy in lupus nephritis. *Arthritis Res Ther*. 2013; **15**: R12. 10.1186/ar4142.

7 Keeler E, Fioravanti G, Samuel B, Longo S. Scleroderma renal crisis or thrombotic thrombocytopenic purpura: seeing through the masquerade. *Lab Med*. 2015; **46**: e39-44. 10.1309/lm72am5xfhzyoqcb.

8 Turner RC, Chaplinski TJ, Adams HG. Rocky Mountain spotted fever presenting as thrombotic thrombocytopenic purpura. *Am J Med*. 1986; **81**: 153-7.

9 Morton JM, George JN. Microangiopathic Hemolytic Anemia and Thrombocytopenia in Patients With Cancer. *J Oncol Pract*. 2016; **12**: 523-30. 10.1200/jop.2016.012096.

10 Scully M, Hunt BJ, Benjamin S, Liesner R, Rose P, Peyvandi F, Cheung B, Machin SJ, British Committee for Standards in H. Guidelines on the diagnosis and management of thrombotic thrombocytopenic purpura and

other thrombotic microangiopathies. *Br J Haematol*. 2012; **158**: 323-35. 10.1111/j.1365-2141.2012.09167.x.

11 Coppo P, Wolf M, Veyradier A, Bussel A, Malot S, Millot GA, Daubin C, Bordessoule D, Pene F, Mira JP, Heshmati F, Maury E, Guidet B, Boulanger E, Galicier L, Parquet N, Vernant JP, Rondeau E, Azoulay E, Schlemmer B, Reseau d'Etude des Microangiopathies Thrombotiques de lA. Prognostic value of inhibitory anti-ADAMTS13 antibodies in adult-acquired thrombotic thrombocytopenic purpura. *Br J Haematol*. 2006; **132**: 66-74. 10.1111/j.1365-2141.2005.05837.x.

12 Loirat C, Veyradier A, Girma JP, Ribba AS, Meyer D. Thrombotic thrombocytopenic purpura associated with von Willebrand factor-cleaving protease (ADAMTS13) deficiency in children. *Semin Thromb Hemost*. 2006; **32**: 90-7. 10.1055/s-2006-939764.

13 Veyradier A, Obert B, Haddad E, Cloarec S, Nivet H, Foulard M, Lesure F, Delattre P, Lakhdari M, Meyer D, Girma JP, Loirat C. Severe deficiency of the specific von Willebrand factor-cleaving protease (ADAMTS 13) activity in a subgroup of children with atypical hemolytic uremic syndrome. *J Pediatr*. 2003; **142**: 310-7.

14 Wu N, Liu J, Yang S, Kellett ET, Cataland SR, Li H, Wu HM. Diagnostic and prognostic values of ADAMTS13 activity measured during daily plasma exchange therapy in patients with acquired thrombotic thrombocytopenic purpura. *Transfusion (Paris)*. 2014. 10.1111/trf.12762.

15 Jin M, Casper TC, Cataland SR, Kennedy MS, Lin S, Li YJ, Wu HM. Relationship between ADAMTS13 activity in clinical remission and the risk of TTP relapse. *Br J Haematol*. 2008; **141**: 651-8. 10.1111/j.1365-2141.2008.07107.x.

16 Westwood JP, Webster H, McGuckin S, McDonald V, Machin SJ, Scully M. Rituximab for thrombotic thrombocytopenic purpura: benefit of early administration during acute episodes and use of prophylaxis to prevent relapse. *J Thromb Haemost*. 2013; **11**: 481-90. 10.1111/jth.12114.

17 Scully M, Cohen H, Cavenagh J, Benjamin S, Starke R, Killick S, Mackie I, Machin SJ. Remission in acute refractory and relapsing thrombotic thrombocytopenic purpura following rituximab is associated with a reduction in IgG antibodies to ADAMTS-13. *Br J Haematol*. 2007; **136**: 451-61. 10.1111/j.1365-2141.2006.06448.x.

18 Hie M, Gay J, Galicier L, Provot F, Presne C, Poullin P, Bonmarchand G, Wynckel A, Benhamou Y, Vanhille P, Servais A, Bordessoule D, Coindre

JP, Hamidou M, Vernant JP, Veyradier A, Coppo P. Preemptive rituximab infusions after remission efficiently prevent relapses in acquired thrombotic thrombocytopenic purpura. *Blood*. 2014; **124**: 204-10. 10.1182/blood-2014-01-550244.

19 Lotta LA, Garagiola I, Palla R, Cairo A, Peyvandi F. ADAMTS13 mutations and polymorphisms in congenital thrombotic thrombocytopenic purpura. *Hum Mutat*. 2010; **31**: 11-9. 10.1002/humu.21143.

20 Kokame K, Matsumoto M, Soejima K, Yagi H, Ishizashi H, Funato M, Tamai H, Konno M, Kamide K, Kawano Y, Miyata T, Fujimura Y. Mutations and common polymorphisms in ADAMTS13 gene responsible for von Willebrand factor-cleaving protease activity. *Proc Natl Acad Sci U S A*. 2002; **99**: 11902-7. 10.1073/pnas.172277399.

21 Levy GG, Nichols WC, Lian EC, Foroud T, McClintick JN, McGee BM, Yang AY, Siemieniak DR, Stark KR, Gruppo R, Sarode R, Shurin SB, Chandrasekaran V, Stabler SP, Sabio H, Bouhassira EE, Upshaw JD, Jr., Ginsburg D, Tsai HM. Mutations in a member of the ADAMTS gene family cause thrombotic thrombocytopenic purpura. *Nature*. 2001; **413**: 488-94. 10.1038/35097008.

22 Fujimura Y, Matsumoto M, Isonishi A, Yagi H, Kokame K, Soejima K, Murata M, Miyata T. Natural history of Upshaw-Schulman syndrome based on ADAMTS13 gene analysis in Japan. *J Thromb Haemost*. 2011; **9 Suppl 1**: 283-301. 10.1111/j.1538-7836.2011.04341.x.

23 Scully M, Starke R, Lee R, Mackie I, Machin S, Cohen H. Successful management of pregnancy in women with a history of thrombotic thrombocytopaenic purpura. *Blood Coagul Fibrinolysis*. 2006; **17**: 459-63. 10.1097/01.mbc.0000240918.65306.20.

24 Moatti-Cohen M, Garrec C, Wolf M, Boisseau P, Galicier L, Azoulay E, Stepanian A, Delmas Y, Rondeau E, Bezieau S, Coppo P, Veyradier A. Unexpected frequency of Upshaw-Schulman syndrome in pregnancy-onset thrombotic thrombocytopenic purpura. *Blood*. 2012; **119**: 5888-97. 10.1182/blood-2012-02-408914.

25 von Krogh AS, Kremer Hovinga JA, Tjonnfjord GE, Ringen IM, Lammle B, Waage A, Quist-Paulsen P. The impact of congenital thrombotic thrombocytopenic purpura on pregnancy complications. *Thromb Haemost*. 2014; **111**: 1180-3. 10.1160/th13-08-0713.

26 Furlan M, Robles R, Morselli B, Sandoz P, Lammle B. Recovery and half-life of von Willebrand factor-cleaving protease after plasma therapy in

patients with thrombotic thrombocytopenic purpura. *Thromb Haemost*. 1999; **81**: 8-13.

27 Scully M, Brown J, Patel R, McDonald V, Brown CJ, Machin S. Human leukocyte antigen association in idiopathic thrombotic thrombocytopenic purpura: evidence for an immunogenetic link. *J Thromb Haemost*. 2010; **8**: 257-62. 10.1111/j.1538-7836.2009.03692.x.

28 Hart D, Sayer R, Miller R, Edwards S, Kelly A, Baglin T, Hunt B, Benjamin S, Patel R, Machin S, Scully M. Human immunodeficiency virus associated thrombotic thrombocytopenic purpura--favourable outcome with plasma exchange and prompt initiation of highly active antiretroviral therapy. *Br J Haematol*. 2011; **153**: 515-9. 10.1111/j.1365-2141.2011.08636.x.

29 McCarthy LJ, Porcu P, Fausel CA, Sweeney CJ, Danielson CF. Thrombotic thrombocytopenic purpura and simvastatin. *Lancet*. 1998; **352**: 1284-5. 10.1016/s0140-6736(05)70492-x.

30 Thomas MR, McDonald V, Machin SJ, Scully MA. Thrombotic thrombocytopenic purpura associated with statin therapy. *Blood Coagul Fibrinolysis*. 2011; **22**: 762-3. 10.1097/MBC.0b013e32834a6170.

31 Martin MG, Whitlatch NL, Shah B, Arepally GM. Thrombotic thrombocytopenic purpura induced by trimethoprim-sulfamethoxazole in a Jehovah's Witness. *Am J Hematol*. 2007; **82**: 679-81. 10.1002/ajh.20887.

32 Sallee M, Cretel E, Jean R, Chiche L, Bourliere M, Poullin P, Lefevre P, Durand JM. Thrombotic thrombocytopenic purpura complicating interferon therapy in chronic C hepatitis. *Gastroenterol Clin Biol*. 2008; **32**: 145-6.

33 Roriz M, Landais M, Desprez J, Barbet C, Azoulay E, Galicier L, Wynckel A, Baudel JL, Provot F, Pene F, Mira JP, Presne C, Poullin P, Delmas Y, Kanouni T, Seguin A, Mousson C, Servais A, Bordessoule D, Perez P, Chauveau D, Veyradier A, Halimi JM, Hamidou M, Coppo P. Risk Factors for Autoimmune Diseases Development After Thrombotic Thrombocytopenic Purpura. *Medicine (Baltimore)*. 2015; **94**: e1598. 10.1097/md.0000000000001598.

34 Schattner A, Friedman J, Klepfish A. Thrombotic thrombocytopenic purpura as an initial presentation of primary Sjogren's syndrome. *Clin Rheumatol*. 2002; **21**: 57-9.

35 Malak S, Wolf M, Millot GA, Mariotte E, Veyradier A, Meynard JL, Korach JM, Malot S, Bussel A, Azoulay E, Boulanger E, Galicier L, Devaux E,

Eschwege V, Gallien S, Adrie C, Schlemmer B, Rondeau E, Coppo P. Human immunodeficiency virus-associated thrombotic microangiopathies: clinical characteristics and outcome according to ADAMTS13 activity. *Scand J Immunol*. 2008; **68**: 337-44. 10.1111/j.1365-3083.2008.02143.x.

36 Menne J, Nitschke M, Stingele R, Abu-Tair M, Beneke J, Bramstedt J, Bremer JP, Brunkhorst R, Busch V, Dengler R, Deuschl G, Fellermann K, Fickenscher H, Gerigk C, Goettsche A, Greeve J, Hafer C, Hagenmuller F, Haller H, Herget-Rosenthal S, Hertenstein B, Hofmann C, Lang M, Kielstein JT, Klostermeier UC, Knobloch J, Kuehbacher M, Kunzendorf U, Lehnert H, Manns MP, Menne TF, Meyer TN, Michael C, Munte T, Neumann-Grutzeck C, Nuernberger J, Pavenstaedt H, Ramazan L, Renders L, Repenthin J, Ries W, Rohr A, Rump LC, Samuelsson O, Sayk F, Schmidt BM, Schnatter S, Schocklmann H, Schreiber S, von Seydewitz CU, Steinhoff J, Stracke S, Suerbaum S, van de Loo A, Vischedyk M, Weissenborn K, Wellhoner P, Wiesner M, Zeissig S, Buning J, Schiffer M, Kuehbacher T. Validation of treatment strategies for enterohaemorrhagic Escherichia coli O104:H4 induced haemolytic uraemic syndrome: case-control study. *BMJ*. 2012; **345**: e4565. 10.1136/bmj.e4565.

37 Vieira-Martins P, El Sissy C, Bordereau P, Gruber A, Rosain J, Fremeaux-Bacchi V. Defining the genetics of thrombotic microangiopathies. *Transfus Apher Sci*. 2016; **54**: 212-9. 10.1016/j.transci.2016.04.011.

38 Dragon-Durey MA, Blanc C, Garnier A, Hofer J, Sethi SK, Zimmerhackl LB. Anti-factor H autoantibody-associated hemolytic uremic syndrome: review of literature of the autoimmune form of HUS. *Semin Thromb Hemost*. 2010; **36**: 633-40. 10.1055/s-0030-1262885.

39 Lemaire M, Fremeaux-Bacchi V, Schaefer F, Choi M, Tang WH, Le Quintrec M, Fakhouri F, Taque S, Nobili F, Martinez F, Ji W, Overton JD, Mane SM, Nurnberg G, Altmuller J, Thiele H, Morin D, Deschenes G, Baudouin V, Llanas B, Collard L, Majid MA, Simkova E, Nurnberg P, Rioux-Leclerc N, Moeckel GW, Gubler MC, Hwa J, Loirat C, Lifton RP. Recessive mutations in DGKE cause atypical hemolytic-uremic syndrome. *Nat Genet*. 2013; **45**: 531-6. 10.1038/ng.2590.

40 Bresin E, Rurali E, Caprioli J, Sanchez-Corral P, Fremeaux-Bacchi V, Rodriguez de Cordoba S, Pinto S, Goodship TH, Alberti M, Ribes D, Valoti E, Remuzzi G, Noris M. Combined complement gene mutations in atypical hemolytic uremic syndrome influence clinical phenotype. *J Am Soc Nephrol*. 2013; **24**: 475-86. 10.1681/asn.2012090884.

41 Legendre CM, Licht C, Muus P, Greenbaum LA, Babu S, Bedrosian C, Bingham C, Cohen DJ, Delmas Y, Douglas K, Eitner F, Feldkamp T, Fouque

D, Furman RR, Gaber O, Herthelius M, Hourmant M, Karpman D, Lebranchu Y, Mariat C, Menne J, Moulin B, Nurnberger J, Ogawa M, Remuzzi G, Richard T, Sberro-Soussan R, Severino B, Sheerin NS, Trivelli A, Zimmerhackl LB, Goodship T, Loirat C. Terminal complement inhibitor eculizumab in atypical hemolytic-uremic syndrome. *N Engl J Med*. 2013; **368**: 2169-81. 10.1056/NEJMoa1208981.

42 Nishimura J, Yamamoto M, Hayashi S, Ohyashiki K, Ando K, Brodsky AL, Noji H, Kitamura K, Eto T, Takahashi T, Masuko M, Matsumoto T, Wano Y, Shichishima T, Shibayama H, Hase M, Li L, Johnson K, Lazarowski A, Tamburini P, Inazawa J, Kinoshita T, Kanakura Y. Genetic variants in C5 and poor response to eculizumab. *N Engl J Med*. 2014; **370**: 632-9. 10.1056/NEJMoa1311084.

43 Ariceta G, Besbas N, Johnson S, Karpman D, Landau D, Licht C, Loirat C, Pecoraro C, Taylor CM, Van de Kar N, Vandewalle J, Zimmerhackl LB. Guideline for the investigation and initial therapy of diarrhea-negative hemolytic uremic syndrome. *Pediatr Nephrol*. 2009; **24**: 687-96. 10.1007/s00467-008-0964-1.

44 Miyakawa Y, Imada K, Ichinohe T, Nishio K, Abe T, Murata M, Ueda Y, Fujimura Y, Matsumoto M, Okamoto S. Efficacy and safety of rituximab in Japanese patients with acquired thrombotic thrombocytopenic purpura refractory to conventional therapy. *Int J Hematol*. 2016; **104**: 228-35. 10.1007/s12185-016-2019-x.

45 Toussaint-Hacquard M, Coppo P, Soudant M, Chevreux L, Mathieu-Nafissi S, Lecompte T, Gross S, Guillemin F, Schneider T. Type of plasma preparation used for plasma exchange and clinical outcome of adult patients with acquired idiopathic thrombotic thrombocytopenic purpura: a French retrospective multicenter cohort study. *Transfusion (Paris)*. 2015; **55**: 2445-51. 10.1111/trf.13229.

46 Scully M, McDonald V, Cavenagh J, Hunt BJ, Longair I, Cohen H, Machin SJ. A phase 2 study of the safety and efficacy of rituximab with plasma exchange in acute acquired thrombotic thrombocytopenic purpura. *Blood*. 2011; **118**: 1746-53. 10.1182/blood-2011-03-341131.

47 Peyvandi F, Lavoretano S, Palla R, Feys HB, Vanhoorelbeke K, Battaglioli T, Valsecchi C, Canciani MT, Fabris F, Zver S, Reti M, Mikovic D, Karimi M, Giuffrida G, Laurenti L, Mannucci PM. ADAMTS13 and anti-ADAMTS13 antibodies as markers for recurrence of acquired thrombotic thrombocytopenic purpura during remission. *Haematologica*. 2008; **93**: 232-9. 10.3324/haematol.11739.

48 Scully M, Thomas M, Underwood M, Watson H, Langley K, Camilleri RS, Clark A, Creagh D, Rayment R, McDonald V, Roy A, Evans G, McGuckin S, Ni Ainle F, Maclean R, Lester W, Nash M, Scott R, P OB. Thrombotic thrombocytopenic purpura and pregnancy: presentation, management, and subsequent pregnancy outcomes. *Blood*. 2014; **124**: 211-9. 10.1182/blood-2014-02-553131.

49 Furlan M, Lammle B. Aetiology and pathogenesis of thrombotic thrombocytopenic purpura and haemolytic uraemic syndrome: the role of von Willebrand factor-cleaving protease. *Best Pract Res Clin Haematol*. 2001; **14**: 437-54. 10.1053/beha.2001.0142.

50 Benhamou Y, Assie C, Boelle PY, Buffet M, Grillberger R, Malot S, Wynckel A, Presne C, Choukroun G, Poullin P, Provot F, Gruson D, Hamidou M, Bordessoule D, Pourrat J, Mira JP, Le Guern V, Pouteil-Noble C, Daubin C, Vanhille P, Rondeau E, Palcoux JB, Mousson C, Vigneau C, Bonmarchand G, Guidet B, Galicier L, Azoulay E, Rottensteiner H, Veyradier A, Coppo P. Development and validation of a predictive model for death in acquired severe ADAMTS13 deficiency-associated idiopathic thrombotic thrombocytopenic purpura: the French TMA Reference Center experience. *Haematologica*. 2012; **97**: 1181-6. 10.3324/haematol.2011.049676.

51 Hughes C, McEwan JR, Longair I, Hughes S, Cohen H, Machin S, Scully M. Cardiac involvement in acute thrombotic thrombocytopenic purpura: association with troponin T and IgG antibodies to ADAMTS 13. *J Thromb Haemost*. 2009; **7**: 529-36. 10.1111/j.1538-7836.2009.03285.x.

52 Cataland SR, Yang SB, Witkoff L, Kraut EH, Lin S, George JN, Wu HM. Demographic and ADAMTS13 biomarker data as predictors of early recurrences of idiopathic thrombotic thrombocytopenic purpura. *Eur J Haematol*. 2009; **83**: 559-64. 10.1111/j.1600-0609.2009.01331.x.

53 Thomas MR, de Groot R, Scully MA, Crawley JT. Pathogenicity of Anti-ADAMTS13 Autoantibodies in Acquired Thrombotic Thrombocytopenic Purpura. *EBioMedicine*. 2015; **2**: 940-50. 10.1016/j.ebiom.2015.06.007.

54 Kokame K, Nobe Y, Kokubo Y, Okayama A, Miyata T. FRETS-VWF73, a first fluorogenic substrate for ADAMTS13 assay. *Br J Haematol*. 2005; **129**: 93-100. 10.1111/j.1365-2141.2005.05420.x.

55 Kato S, Matsumoto M, Matsuyama T, Isonishi A, Hiura H, Fujimura Y. Novel monoclonal antibody-based enzyme immunoassay for determining plasma levels of ADAMTS13 activity. *Transfusion (Paris)*. 2006; **46**: 1444-52. 10.1111/j.1537-2995.2006.00914.x.

56 Muia J, Gao W, Haberichter SL, Dolatshahi L, Zhu J, Westfield LA, Covill SC, Friedman KD, Sadler JE. An optimized fluorogenic ADAMTS13 assay with increased sensitivity for the investigation of patients with thrombotic thrombocytopenic purpura. *J Thromb Haemost*. 2013; **11**: 1511-8. 10.1111/jth.12319.

57 Jin M, Cataland S, Bissell M, Wu HM. A rapid test for the diagnosis of thrombotic thrombocytopenic purpura using surface enhanced laser desorption/ionization time-of-flight (SELDI-TOF)-mass spectrometry. *J Thromb Haemost*. 2006; **4**: 333-8. 10.1111/j.1538-7836.2006.01758.x.

58 Hubbard AR, Heath AB, Kremer Hovinga JA. Establishment of the WHO 1st International Standard ADAMTS13, plasma (12/252): communication from the SSC of the ISTH. *J Thromb Haemost*. 2015; **13**: 1151-3. 10.1111/jth.12881.

59 Peyvandi F, Palla R, Lotta LA, Mackie I, Scully MA, Machin SJ. ADAMTS-13 assays in thrombotic thrombocytopenic purpura. *J Thromb Haemost*. 2010; **8**: 631-40. 10.1111/j.1538-7836.2010.03761.x.

60 Meyer SC, Sulzer I, Lammle B, Kremer Hovinga JA. Hyperbilirubinemia interferes with ADAMTS-13 activity measurement by FRETS-VWF73 assay: diagnostic relevance in patients suffering from acute thrombotic microangiopathies. *J Thromb Haemost*. 2007; **5**: 866-7. 10.1111/j.1538-7836.2007.02438.x.

61 Lu RN, Yang S, Wu HM, Zheng XL. Unconjugated bilirubin inhibits proteolytic cleavage of von Willebrand factor by ADAMTS13 protease. *J Thromb Haemost*. 2015; **13**: 1064-72. 10.1111/jth.12901.

62 Mackie I, Langley K, Chitolie A, Liesner R, Scully M, Machin S, Peyvandi F. Discrepancies between ADAMTS13 activity assays in patients with thrombotic microangiopathies. *Thromb Haemost*. 2013; **109**: 488-96. 10.1160/th12-08-0565.

63 Spinale JM, Ruebner RL, Kaplan BS, Copelovitch L. Update on Streptococcus pneumoniae associated hemolytic uremic syndrome. *Curr Opin Pediatr*. 2013; **25**: 203-8. 10.1097/MOP.0b013e32835d7f2c.

64 Sharma AP, Greenberg CR, Prasad AN, Prasad C. Hemolytic uremic syndrome (HUS) secondary to cobalamin C (cblC) disorder. *Pediatr Nephrol*. 2007; **22**: 2097-103. 10.1007/s00467-007-0604-1.

65 Ruutu T, Barosi G, Benjamin RJ, Clark RE, George JN, Gratwohl A, Holler E, Iacobelli M, Kentouche K, Lammle B, Moake JL, Richardson P, Socie G, Zeigler Z, Niederwieser D, Barbui T. Diagnostic criteria for hematopoietic stem cell transplant-associated microangiopathy: results of a consensus process by an International Working Group. *Haematologica*. 2007; **92**: 95-100.

66 Jodele S, Fukuda T, Vinks A, Mizuno K, Laskin BL, Goebel J, Dixon BP, Teusink A, Pluthero FG, Lu L, Licht C, Davies SM. Eculizumab therapy in children with severe hematopoietic stem cell transplantation-associated thrombotic microangiopathy. *Biol Blood Marrow Transplant*. 2014; **20**: 518-25. 10.1016/j.bbmt.2013.12.565.

67 Levi M, Toh CH, Thachil J, Watson HG. Guidelines for the diagnosis and management of disseminated intravascular coagulation. British Committee for Standards in Haematology. *Br J Haematol*. 2009; **145**: 24-33. 10.1111/j.1365-2141.2009.07600.x.

68 Salmon JE, Heuser C, Triebwasser M, Liszewski MK, Kavanagh D, Roumenina L, Branch DW, Goodship T, Fremeaux-Bacchi V, Atkinson JP. Mutations in complement regulatory proteins predispose to preeclampsia: a genetic analysis of the PROMISSE cohort. *PLoS Med*. 2011; **8**: e1001013. 10.1371/journal.pmed.1001013.

69 Martin JN, Jr., Rinehart BK, May WL, Magann EF, Terrone DA, Blake PG. The spectrum of severe preeclampsia: comparative analysis by HELLP (hemolysis, elevated liver enzyme levels, and low platelet count) syndrome classification. *Am J Obstet Gynecol*. 1999; **180**: 1373-84.

70 Zakarija A, Kwaan HC, Moake JL, Bandarenko N, Pandey DK, McKoy JM, Yarnold PR, Raisch DW, Winters JL, Raife TJ, Cursio JF, Luu TH, Richey EA, Fisher MJ, Ortel TL, Tallman MS, Zheng XL, Matsumoto M, Fujimura Y, Bennett CL. Ticlopidine- and clopidogrel-associated thrombotic thrombocytopenic purpura (TTP): review of clinical, laboratory, epidemiological, and pharmacovigilance findings (1989-2008). *Kidney Int Suppl*. 2009: S20-4. 10.1038/ki.2008.613.

71 Kojouri K, Vesely SK, George JN. Quinine-associated thrombotic thrombocytopenic purpura-hemolytic uremic syndrome: frequency, clinical features, and long-term outcomes. *Ann Intern Med*. 2001; **135**: 1047-51.

72 Eremina V, Jefferson JA, Kowalewska J, Hochster H, Haas M, Weisstuch J, Richardson C, Kopp JB, Kabir MG, Backx PH, Gerber HP, Ferrara N, Barisoni L, Alpers CE, Quaggin SE. VEGF inhibition and renal

thrombotic microangiopathy. *N Engl J Med*. 2008; **358**: 1129-36. 10.1056/NEJMoa0707330.

73 McDonald V, Laffan M, Benjamin S, Bevan D, Machin S, Scully MA. Thrombotic thrombocytopenic purpura precipitated by acute pancreatitis: a report of seven cases from a regional UK TTP registry. *Br J Haematol*. 2009; **144**: 430-3. 10.1111/j.1365-2141.2008.07458.x.

74 Swisher KK, Doan JT, Vesely SK, Kwaan HC, Kim B, Lammle B, Kremer Hovinga JA, George JN. Pancreatitis preceding acute episodes of thrombotic thrombocytopenic purpura-hemolytic uremic syndrome: report of five patients with a systematic review of published reports. *Haematologica*. 2007; **92**: 936-43.

75 Lechner K, Obermeier HL. Cancer-related microangiopathic hemolytic anemia: clinical and laboratory features in 168 reported cases. *Medicine (Baltimore)*. 2012; **91**: 195-205. 10.1097/MD.0b013e3182603598.

Disease	Summary of Disease	Comment
TTP	Acute haematological emergency, requiring prompt diagnosis and treatment. More likely to have neurological/cardiac features-but a multi system disorder.	ADAMTS 13 measured for definitive diagnosis. Plasma exchange is the mainstay of treatment, typically additional immunomodulating therapy required in iTTP.

HUS A. IA-HUS	The TMA presents 5-7 days after the infection with haemorrhagic colitis.	Confirmation of infection is serological and/PCR.
B CM-HUS	CM-HUS can be triggered by infection (and diarrhoea maybe present at presentation), vaccinations, or pregnancy. .	Diagnosis may be confirmed by complement mutations eg CFH, CFI, MCP, CFB, C3 or anti CFH antibodies. Homozygous or compound heterozygous diacylglycerol kinase (DGKE) mutations typically do not respond to current complement inhibitors [39] Treatment decisions must be made on the basis of the clinical presentation and absence of severe ADAMTS13 deficiency. A response to anti-complement therapy may provide

		confirmation of the diagnosis.
C. Streptococcus pneumoniae	This is seen almost exclusively in young children. Typically present with pneumonia. Incidence reduced since polyvalent vaccine (in some countries). Have a DAT positive haemolytic anaemia	Thomsen-Friedenreich cryptoantigen, present on red cells, platelet, glomeruli and hepatocytes are exposed by neuramindase, produced by all subtypes of Streptococcus pneumoniae. The TF antigen interacts with IgM antibodies, resulting in agglutination. Treatment is supportive, but still washed blood products may be requested [63]

D. Cobalamin C deficiency	A rare cause of HUS that is caused by recessive mutations of the methylmalonic aciduria (cobalamin deficiency) cblC type, with homocystinuria (MMACHC) gene. Presentation is typically within the first year of life [64].	
Connective tissue disease	MAHAT may be a primary feature. Further systemic features/laboratory testing confirm the diagnosis	Autoimmune screen, tissue biopsy eg SLE, scleroderma, antiphospholipid syndrome, vasculitis (eg ANCA)

Transplantation, either solid organ or bone marrow/ hematopoietic stem cell (HSC):	May present with MAHAT with or without renal involvement. It is typically associated with immunosuppressive therapy, such as Tacrolimus, Ciclosporin A, underlying opportunistic infections (eg CMV, Adenovirus, Aspergillus) or graft-versus-host disease [65],	HSC transplant associated TMAs do not have severe ADAMTS13 deficiency (ie <10%). Complement alternative pathway dysregulation may be involved in the pathogenesis of HSCT-TMA . In patients developing TMA post renal transplantation, consideration should be given to possible CM-HUS, which may have been the cause of the initial renal failure and the failed transplanted kidney [66]
Disseminated intravascular Coagulation (DIC)	DIC is associated with abnormalities in routine coagulation and CBC screens [67].	DIC is typically due to an underlying precipitant such as sepsis, malignancy, haematological disorders, obstetric complications or trauma.

Pregnancy associated TMAS	Preeclampsia and HELLP occur in the second half of pregnancy or early post partum.	Complement activation in HELLP or preeclampsia may trigger the development of TMA. Complement abnormalities have been identified in some cases of HELLP/PE [68],
A. HELLP syndrome	There are no specific diagnostic clinical or laboratory factors. ADAMTS13 activity is typically normal. HELLP can be divided into type 1, 2 and 3 based on the platelet count ($\leq$50 x10^9/L, 50-100 x10^9/L, 100-150 x10^9/L) [69].	May be associated with complications such as DIC, and placental abruption.

B. Pre-eclampsia	Defined as de novo hypertension (>140/90 mm Hg) and proteinuria (>0.3 g per 24 hours) after 20 weeks of gestation .	Control of BP. Delivery will improve the situation.
Drugs	Ticlodipine [70] may be associated with autoantibodies to ADAMTS 13, case reports with quinine [71], simvastatin, trimethoprim or interferon. Clopidogrel is not associated with TTP. TMA may occur with gemcitabine, bleomycin, and mitomycin. VEGF inhibitors were associated with features of TMA reminiscent of HUS [72].	Stopping the offending drug maybe associated with improvement.

Pancreatitis	Caused by alcohol excess, bile duct obstruction or possibly an autoimmune precipitant [73].	ADAMTS13 levels may not be severely reduced [74]. Identifying the underlying condition reduces the risk of further relapses.
Malignancy	In patients with MAHAT for whom a severe deficiency of ADAMTS13 activity is not confirmed, tumour markers and radiological investigations should be considered [75].	ADAMTS13 activity is normal or mildly decreased.

Table 1: Summary of characteristics associated with conditions presenting with microangiopathic haemolytic anaemia and thrombocytopenia (MAHAT)

IR-HUS: Infection related haemolytic uraemic syndrome; PEX: plasma exchange, BP: blood pressure, HELLP: haemolysis, elevated liver enzymes & low platelets, DIC: disseminated intravascular coagulation, TMA: thrombotic microangiopathy, CM-HUS: . Complement mediated-HUS

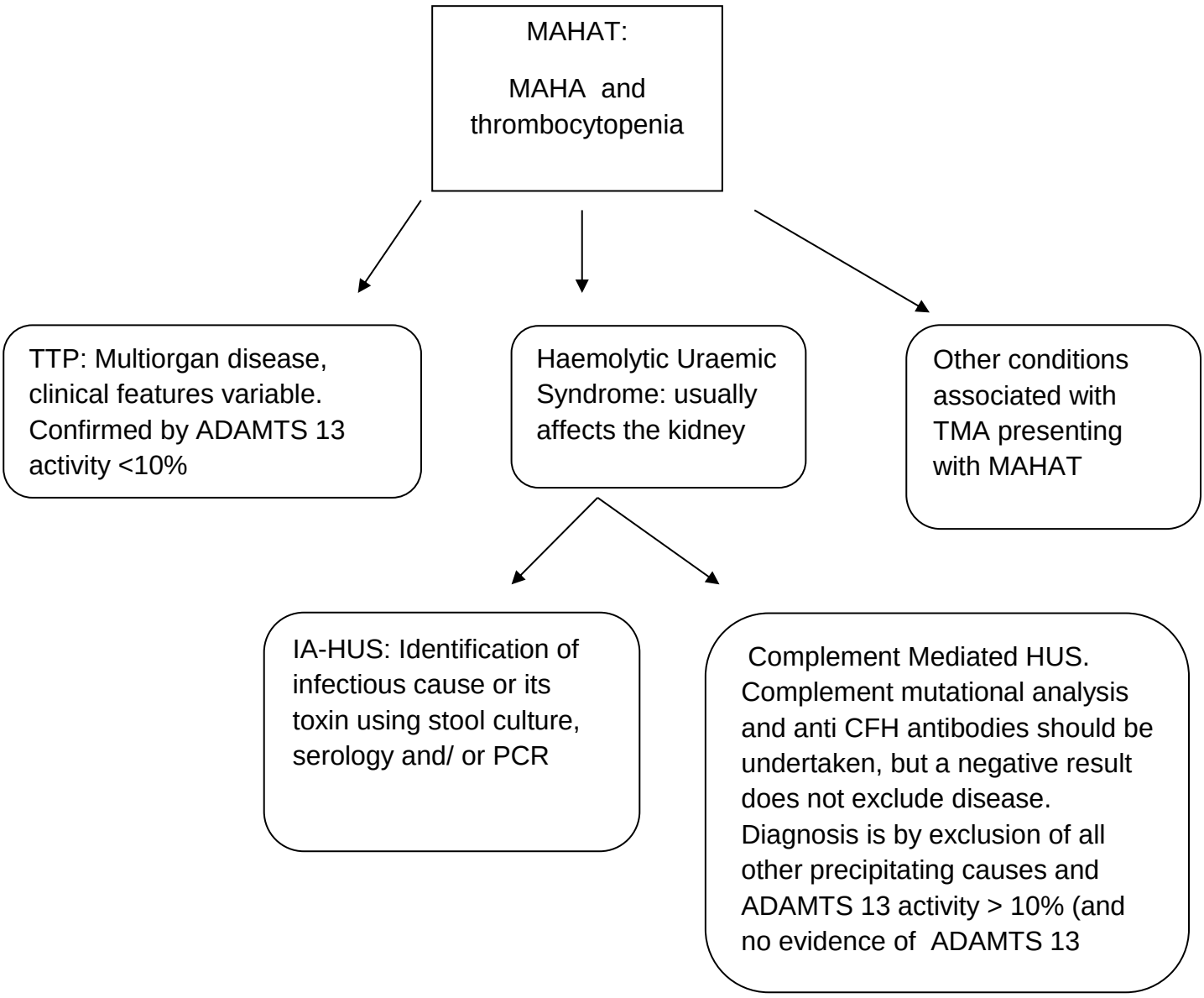

Figure 1: Differential diagnosis of thrombotic Microangiopathies presenting with MAHAT

Key: MAHA: microangiopathic haemolytic anaemia; MAHAT: MAHA and thrombocytopenia-TTP: thrombotic thrombocytopenic purpura; IA-HUS: Infection associated HUS, PCR: polymerase chain reaction